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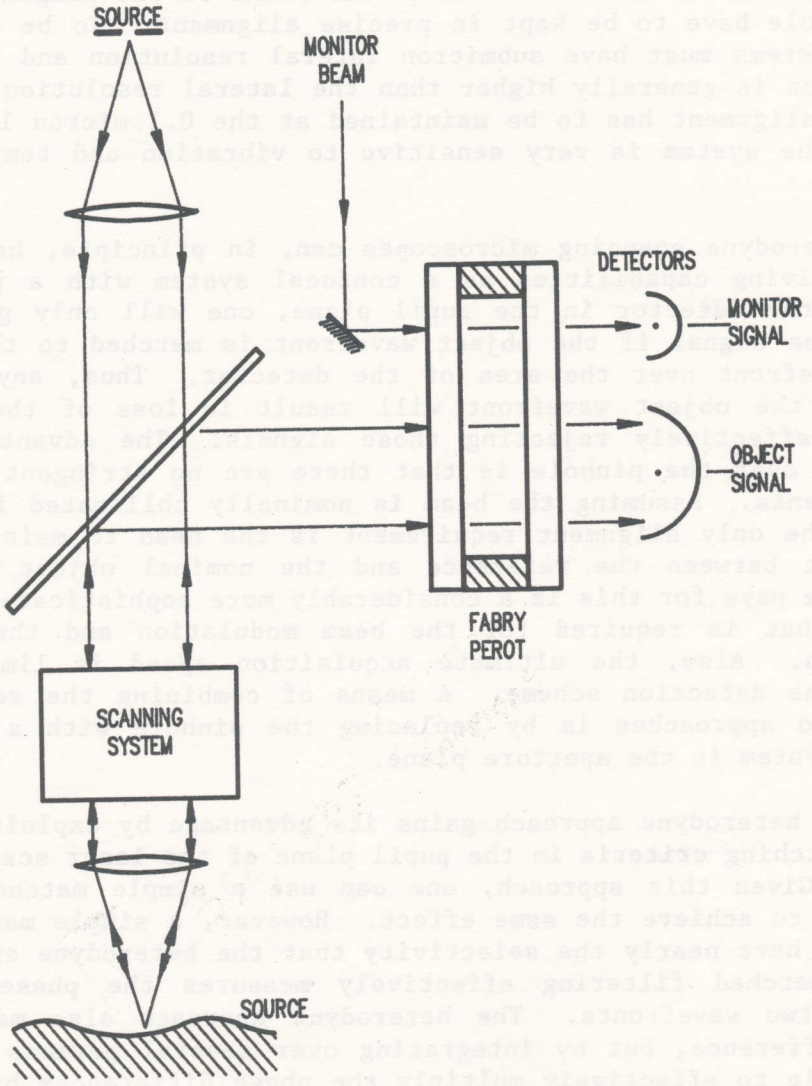
CONFOCAL MICROSCOPE WITH FABRY-PEROT FILTER



A confocal laser scanning microscope is described wherein the conventional pinhole is replaced with one or more Fabry-Perot filters to achieve performance improvement.

In a conventional confocal laser scanning microscope, resolution gain is obtained by first illuminating the object with a diffraction limited spot, and then imaging that spot onto a pinhole. The pinhole

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diameter is set at the diffraction limit of the system. Thus, stray light from outside the diffraction limited spot is prevented from reaching the detector, thus increasing the lateral resolution. Similarly, if the object is out of focus, a blurred spot is formed in the image plane and most of the light is rejected by the pinhole. Thus, the detector only sees the in-focus plane. These two features open up unique applications for confocal microscopes. A major difficulty in constructing and maintaining a confocal system is the stringent mechanical tolerances involved. The focal point of the imaging system and the pinhole have to be kept in precise alignment. To be of interest, these systems must have submicron lateral resolution and the vertical resolution is generally higher than the lateral resolution. Thus, the pinhole alignment has to be maintained at the 0.1 micron level. Obviously, the system is very sensitive to vibration and temperature effects.

Heterodyne scanning microscopes can, in principle, have the same 3-D resolving capabilities as a confocal system with a pinhole. By placing the detector in the pupil plane, one will only get a strong heterodyne signal if the object wavefront is matched to the reference beam wavefront over the area of the detector. Thus, any defocus or tilt in the object wavefront will result in loss of the heterodyne signal, effectively rejecting those signals. The advantage of this approach over the pinhole is that there are no stringent positioning requirements. Assuming the beam is nominally collimated in the pupil plane, the only alignment requirement is the need to maintain angular alignment between the reference and the nominal object beams. The price one pays for this is a considerably more sophisticated electronic system that is required for the beam modulation and the heterodyne detection. Also, the ultimate acquisition speed is limited by the heterodyne detection scheme. A means of combining the advantages of these two approaches is by replacing the pinhole with a Fabry-Perot filter system in the aperture plane.

The heterodyne approach gains its advantage by exploiting a wavefront matching criteria in the pupil plane of the laser scanning microscope. Given this approach, one can use a simple matched filtering approach to achieve the same effect. However, a simple matched filter does not have nearly the selectivity that the heterodyne approach has. Simple matched filtering effectively measures the phase difference between two wavefronts. The heterodyne approach also measures this phase difference, but by integrating over several periods temporally, it is able to effectively multiply the phase differences by the number of integration periods. The number of integration periods may typically be 1000 or more, thus attaining very high sensitivity. A Fabry-Perot is effectively a matched filter (matched for a plane wave with a given k vector) which also attains very high sensitivity by employing multiple interference. The multiple interference multiplies the phase differences by the number of interfering beams, or the effective number

of passes of a beam through the Fabry-Perot. This number can also be on the order of 1000 or more. Fabry-Perot filters are normally used as spectral filters, but here the wavelength can be kept fixed and the Fabry-Perot can be a filter sensitive to angle of incidence and beam collimation. When necessary, the Fabry-Perot can be actively controlled by monitoring the transmission of a second reference beam from the laser in order to compensate for thermal drift, etc. The effective cavity length of the filter can also be modulated. This allows AC detection to improve signal-to-noise, if necessary, and it also scans the acceptance angle of the filter which would allow one to obtain both normal "brightfield" data as well as selective "darkfield" data. This centrosymmetric darkfield mode is not feasible with a normal confocal system. The Fabry-Perot can be easily designed to have an angular selectivity of 0.1 milliradians. An 0.1-milliradian selectivity corresponds to a confocal microscope with a 1-micron pinhole behind a 1-cm focal length lens. Thus, the selectivity of a typical confocal system is easily obtained without the 3-D position alignment requirements.

The figure shows one implementation of the system. The optics consist of the normal laser scanning system for projection of the scanning point. In the diagram, the incident wave and object signal correspond to a wave at normal incidence to the Fabry-Perot filter. Alternatively, two filters can be substituted for the one shown, with the light from the object propagating through both. The two Fabry-Perots would be identical, but tipped at an angle to the central ray from the object in orthogonal planes. In the imaging part, the Fabry-Perot is placed in the exit pupil plane and the detector integrates the output from the Fabry-Perot.